SPORULATION OF *PYRICULARIA* SPP. ON DETACHED LEAVES OF GRAMINEAE AND IN MEDIA SUPPLEMENTED WITH LEAF EXTRACTS*

BY

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ABSTRACT

Except for the isolate from *S. italica*, all other isolates of *Pyricularia* sporulated best on detached injured leaves of *T. vulgare* and *S. italica* as compared to their natural host leaves in light. The observed results are ascribed to heterokaryosis and production/selection of sporulating biotypes rather than to the stimulation of sporulation *per se* of the isolates by special pre- or post-inoculation compounds in the detached leaves.

INTRODUCTION

THE frequent failure to obtain good sporulation of *Pyricularia* spp. in culture is well recognized. This is particularly true of isolates of *Pyricularia* obtained from the grasses *Panicum repens*, *Brachiaria mutica* and *Leersia hexandra* (Narayanarao *et al.*, 1972 *a*, *b*). Among the three grass isolates, that from *B. mutica* seldom sporulates on a variety of defined, semi-synthetic and natural media. The other two isolates sporulate poorly on many media. In attempting to induce sporulation in a non-sporulating isolate from *B. mutica* by various means, it was found that inoculation of injured detached leaf bits of *B. mutica* with the isolate resulted in conidial induction when the leaf bits were kept under light. It was, therefore, of interest to study conidial production by other isolates of *Pyricularia* on detached leaves of their natural hosts, and when they were cross-inoculated on detached leaves. In this study, leaves from *Avena sativa*, *Triticum vulgare*, *Hordeum vulgare* and *Secale cereale* were also used since all *Pyricularia* spp. under report have been demonstrated to successfully infect these plants (Thiagarajan, 1970). Further, the effect of leaf extracts of some of the gramineous plants on sporulation of *Pyricularia* spp. in culture is reported here.

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MATERIALS AND METHODS

The materials used, cultural and other methods were as described previously (Narayanarao *et al.*, 1972 *a*).

Source of detached leaves.—Detached leaves were obtained from *Oryza sativa* (CO 13), *Eleusine coracana* (CO 5), *Setaria italica* (CO 1), *Panicum repens*, *Brachiaria mutica*, *Leersia hexandra*, *Triticum vulgare* (NP 200), *Hordeum vulgare* (Russian), *Secale cereale* and *Avena sativa* (EC 35162).

The plants were raised in gravel culture under controlled conditions in a growth chamber under fluorescent and incandescent lighting giving 9,500 to 10,000 Lux near the plants. The duration of the light period was 12 hr. The temperature during the light period was $30 \pm 2°$ C. The nyctotemperature of the growth room was maintained at $20 \pm 1°$ C. Arnon and Hoagland (1940) nutrient solution and distilled water were supplied to the plants on alternate days. The cultivated species of Gramineae were raised from seeds and the grasses from stem cuttings containing two nodes.

The plants were grown for one month and the middle portion of the fully expanded first leaves were cut into 2·0 cm bits. They were washed with glass-distilled water and then thrice with sterile glass-distilled water. The upper surface of the leaf bit was injured by scrapping from one end to the other with a sterile stainless steel spatula. A small mycelial agar block from a 10–12 day old culture maintained on potato-sucrose agar was smeared on the injured leaf surface. Care was taken to use the same amount of inoculum. Duplicate leaf bits for each treatment were kept on microscope slides in a Petri dish moist chamber. They were exposed to 12 hr. light from two 80 W mercury vapour lamps (Phillips) suspended at a height of 20″ from the moist chambers. The dark treatment was achieved by placing them in a totally darkened chamber. The leaf bits were incubated in the growth chamber itself and were thus exposed to the same ambient temperatures prevailing in the growth room.

Aqueous leaf extracts (20%) were prepared by homogenizing the leaves in Waring blender, filtering the homogenate through three layers of muslin cloth and centrifuging the filtrate at 4,000 r.p.m. for 15 min. The clear supernatant was used as extract. Leaf extract agar (2%) was prepared by mixing equal volumes of leaf extract and double strength water agar or sucrose-nitrate medium (Suryanarayanan, 1958). Autoclaving was done at 15–lb./sq.″ for 15 min. When aseptically added, the extract was cold sterilized through a Millipore filter (0·22 μ). Slants of uniform angle were

made with 5 ml of respective media in 15·0 × 1·5 cm test tubes and inoculated at the centre with a pin-point inoculum. The tubes were incubated in the growth chamber under a bank of twelve 40 W (3,400 K) fluorescent tube lights at a distance of 20″ from the lamps. The tubes were exposed to light for a period of 12 hr. each day.

Experimental and Results

Sporulation of Pyricularia *spp. on detached leaves.*—Initially a non-sporulating culture of *Pyricularia* sp. from *B. mutica* was inoculated on detached, injured and uninjured host leaf bits. As evident from the results presented in Table I, the isolate failed to sporulate in uninjured leaf bits. Hence, in subsequent experiments, injured leaf bits were used.

Table I

Sporulation of Pyricularia *sp. from* B. mutica *on* B. mutica *leaf bits*

Days of incubation	No. of conidia/2·0 cm leaf bit (×10⁴)	
	Injured leaf bit	Uninjured leaf bit
4	4	0
6	6	0
8	7	0
10	5	0
12	9	0

Table II

Sporulation of Pyricularia *spp. on their respective injured host leaf bits in light and darkness*

Treatment	Days of incubation	Number of conidia/2·0 cm leaf bit (×10⁴)					
		Isolate from					
		O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
12 h light and 12 h darkness	4	2	*	2	*	3	*
	8	4	3	110	*	7	*
	12	10	5	140	*	12	*
Total darkness	4	*	0	4	0	*	0
	8	3	0	54	0	*	0
	12	6	0	78	0	*	0

* Sparse sporulation. 0: Nil sporulation.

Table II shows the sporulation of *Pyricularia* spp. on their respective host leaf bits exposed to 12 hr light or when kept under total darkness. The isolate from *S. italica* sporulated best under these conditions as compared to the other isolates. The isolates from *P. repens* and *L. hexandra* sporulated very sparsely on their natural host leaf bits. In total darkness, the isolates either failed to sporulate or sporulation was much reduced. In the following experiments, the cultures were, therefore, exposed to a 12 hr light period.

Table III shows sporulation of *Pyricularia* spp. when they were cross-inoculated on injured leaf bits of their natural host plants and also when inoculated on certain other Gramineae, demonstrated to be susceptible to all the isolates of *Pyricularia* included in the present study. The isolate from *L. hexandra* sporulated only sparsely on detached leaves of its natural host. This substratum did not support sporulation of other isolates. The isolates from *E. coracana*, *B. mutica* and *L. hexandra* did not sporulate on detached leaves of *P. repens* and the other isolates sporulated only sparsely. On the other hand, all the isolates except the one from *L. hexandra* sporulated to a certain extent on *B. mutica*. But for the isolates from *O. sativa* and *E. coracana*, the other isolates either did not sporulate or sporulated only sparsely on detached leaves of *O. sativa*. The isolates from the three grasses did not sporulate on *E. coracana* but the isolate from *S. italica* sporulated well on this substratum. However, the isolates from *O. sativa* and *E. coracana* sporulated poorly on these leaves. In contrast to the above results, the detached leaves of *S. italica* supported good sporulation of all the isolates except the one from *L. hexandra* which did not sporulate. Among the other cultivated spp. of Gramineae, *T. vulgare* was the best in supporting good sporulation of all the isolates. It may, however, be noted that the isolates from *E. coracana* and *S. italica* sporulated better on *S. italica* than on *T. vulgare*.

Sporulation of Pyricularia *spp. on agar media supplemented with leaf extracts*.—It is evident from the results presented in Table III that, in general, detached leaves of *P. repens* did not favour sporulation of the isolates of *Pyricularia* tested but five of the six isolates sporulated on *B. mutica*. Sporulation of the isolates was, therefore, next studied on agar media supplemented with heat/cold sterilized leaf extracts of *P. repens* and *B. mutica*. The results are presented in Tables IV and V.

It will be seen from Table IV that neither heat-sterilized nor cold-sterilized leaf extracts favoured sporulation of the isolates from *P. repens* and *B. mutica* on the agar medium. Again, the *P. repens* extract had little effect

TABLE III

Sporulation of Pyricularia *spp. on injured leaf bits of different Gramineae*

Isolates from	Days of incubation	Number of conidia/2·0 cm leaf bit ($\times 10^4$)									
		O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*	*T. vulgare*	*S. cereale*	*H. vulgare*	*A. sativa*
O. sativa	4	2	0	6	*	2	0	16	3	3	2
	8	4	*	30	*	4	0	40	4	4	4
	12	10	*	37	*	8	0	59	10	10	6
E. coracana	4	1	*	3	0	6	0	3	2	*	*
	8	10	3	22	0	12	0	9	4	*	*
	12	20	5	22	0	10	0	5	6	4	6
S. italica	4	*	4	2	0	*	0	4	*	*	*
	8	*	20	110	*	20	0	75	4	4	4
	12	*	16	140	*	15	0	80	12	10	14
P. repens	4	0	0	3	*	*	0	7	5	*	*
	8	0	0	16	*	*	0	19	4	4	4
	12	0	0	22	*	3	0	48	6	6	4
B. mutica	4	*	0	5	0	3	0	26	3	*	2
	8	*	0	9	0	7	0	42	4	*	4
	12	*	0	26	0	12	0	48	8	3	4
L. hexandra	4	0	0	0	0	0	*	3	*	*	*
	8	0	0	0	0	0	*	16	4	*	4
	12	0	0	0	0	0	*	18	6	*	4

*: Sparse sporulation.

0: Nil sporulation.

on sporulation of the isolates from *L. hexandra* and *E. coracana* but the *B. mutica* extract promoted sporulation of these isolates. The isolates from *O. sativa* and *S. italica* sporulated well in the presence of both leaf extracts. In general, the method of sterilization did not markedly affect sporulation.

TABLE IV

Sporulation of Pyricularia *spp. on* P. repens *and* B. mutica *leaf extract agar*

Media	Days of incubation	No. of conidia/tube × 10^4					
		Isolate from					
		O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
Water agar (Control)	4	0	0	0	0	0	0
	8	0	0	0	0	0	0
	12	0	0	0	0	0	0
	16	0	0	0	0	0	0
Autoclaved *P. repens* extract	4	8	3	58	0	0	*
	8	11	3	83	0	0	*
	12	40	6	90	0	0	*
	16	36	4	50	0	0	*
Aseptically added *P. repens* extract	4	8	*	15	0	0	*
	8	55	3	48	*	0	*
	12	70	6	75	*	0	4
	16	44	3	60	*	0	3
Autoclaved *B. mutica* extract	4	26	12	10	0	0	8
	8	70	52	110	0	0	30
	12	30	22	85	0	0	21
	16	22	15	40	0	0	12
Aseptically added *B. mutica* extract	4	25	4	20	0	0	5
	8	70	55	127	0	0	32
	12	80	50	70	0	0	20
	16	50	12	60	0	0	10

* Sparse sporulation. 0: Nil sporulation.

Data on sporulation of the isolates of *Pyricularia* in sucrose-nitrate agar medium supplemented with the leaf extracts are given in Table V. It may be seen that the isolate from *B. mutica* failed to sporulate in any treatment. The isolate from *P. repens* sporulated poorly on all media. The *P. repens* extract did not stimulate sporulation of the isolates from *P. repens* and *L. hexandra*. However, maximum sporulation of these two isolates was obtained with aseptically added *B. mutica* extract. The isolates from *O. sativa*, *E. coracana* and *S. italica* sporulated abundantly in most of the media as compared to the isolates from the three grasses. Aseptically added *B. mutica* extract promoted maximum sporulation of the isolate from *O. sativa*.

On the other hand aseptically added *P. repens* extract was the most beneficial for sporulation of the isolates from *E. coracana* and *S. italica*. It may be further noted that the heat-sterilized *P. repens* extract tended to reduce sporulation of all the isolates except that from *S. italica* as compared to the control.

TABLE V

Sporulation of Pyricularia *spp. in sucrose-nitrate medium supplemented with* P. repens *and* B. mutica *leaf extracts*

Media	Days of incubation	No. of conidia/tube ×10⁴ — Isolate from					
		O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
Sucrose-nitrate medium (SN) (control)	4	28	*	10	*	0	4
	8	120	55	90	3	0	11
	12	170	70	150	3	0	22
	16	133	42	90	*	0	15
S.N. + *P. repens* extract (autoclaved)	4	23	10	120	0	0	2
	8	65	10	225	0	0	5
	12	140	23	210	*	0	6
	16	120	21	180	*	0	4
S.N. + *P. repens* extract (aseptically added)	4	312	180	314	*	0	*
	8	340	210	664	4	0	4
	12	410	320	1080	6	0	10
	16	345	300	884	3	0	7
S.N. + *B. mutica* extract (autoclaved)	4	60	33	53	*	0	5
	8	245	118	332	*	0	93
	12	490	140	230	*	0	70
	16	266	122	115	0	0	62
S.N. + *B. mutica* extract (aseptically added)	4	136	26	71	4	0	15
	8	675	196	262	8	0	120
	12	550	190	374	14	0	150
	16	390	112	253	12	0	96

* Sparse sporulation. 0: Nil sporulation.

The effect of leaf extracts of *T. vulgare* and *S. italica* on sporulation of the isolates of *Pyricularia* was next studied in sucrose-nitrate medium since all but one of the isolates sporulated on detached leaves of these two species of Gramineae (*vide* Table III). The leaf extracts were added to the medium before autoclaving. It will be seen from Table VI that the isolate from *B. mutica* failed to sporulate on all media tried and the isolate from *P. repens*

sporulated poorly. Addition of the leaf extracts tended to reduce sporulation of the isolates from *O. sativa* and *P. repens* but stimulated sporulation of the other isolates.

TABLE VI

Sporulation of Pyricularia *spp. in different media at* 12 *days incubation*

Media	No. of conidia/tube $\times 10^4$					
	Isolate from					
	O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
Sucrose nitrate medium (SN)	190	82	130	10	0	16
S.N. +20% *T. vulgare* extract	86	170	290	6	0	45
S.N. +20% *S. italica* extract	24	103	195	5	*	26
Potato-sucrose agar + biotin and thiamine	370	98	195	*	0	16
Oatmeal agar + biotin and thiamine	380	84	310	*	0	16

* Sparse sporulation. 0: Nil sporulation.

DISCUSSION

It is obvious from the data presented that the isolate of *Pyricularia* from *B. mutica* failed to sporulate consistently on various agar media including those supplemented with leaf extracts (Tables IV, V and VI). The same isolate, however, sporulated on detached-injured leaves of *B. mutica*, *S. italica* and profusely on *T. vulgare* leaves (Table III). It is also evident that the isolate sporulated on injured but not on uninjured detached leaves (Table I). Microscopic examination of inoculated injured and uninjured leaf bits showed that while the fungus entered and colonized the leaf tissue with facility before bearing conidia in the former case, it did not grow out of the inoculated point in the latter. While wounding would appear to facilitate entry and colonization of the substratum by the fungus, the beneficial effect of pre-or post-inoculation compounds of the leaf tissue on the sporulation process cannot be ruled out. Besides injury, light appears to promote sporulation of the isolates on detached leaves (Table II). This is possibly related to photo destruction of compounds inhibitory for sporulation or formation of stimulatory compounds favouring sporulation. In discussing the effect of light on sporulation by *P. oryzae* in culture, Chakrabarthi and Wilcoxson (1970) concluded that light was not necessary for

sporulation and positive light effects were temperature-dependant. However, a more critical study of the effect of light on sporulation of *Pyricularia* both in nature and culture would seem to be warranted.

Inspection of Tables IV, V and VI would further reveal that the isolates from the grasses did not either sporulate or sporulated only poorly on the agar media tried as compared to the isolates from the cultivated cereals. It is also evident that the isolates from *O. sativa* and *S. italica* sporulated better than the isolate from *E. coracana*. These differences among the isolates to sporulate are indeed a reflection of differences in their genetic composition. That all the six isolates sporulated on *T. vulgare* leaf bits and all but one sporulated on *S. italica* leaf bits (Table III) lends further support to this view. *A priori* it would appear that these two substrates contain special compounds that favoured sporulation. However, we have evidence that the grass isolates when passed through intact *T. vulgare* plants infect many more international rice differentials than the parent isolates indicating production and/or selection of new biotypes (Thiagarajan, 1970). It seems, therefore, unlikely that the detached leaves favoured sporulation of the inoculated isolates *per se* but had possibly favoured selection of new sporulating biotypes.

Loss of the ability to sporulate in culture by *Cercospora* isolates has been explained in terms of heterokaryosis (Goode and Brown, 1970). Stable sporulating isolates are believed to be homokaryotic for nuclei containing genes for sporulation. Failure to form conidia in culture has been assumed to be associated with vegetative genes. Mutation for non-sporulation in a single nucleus could establish a heterokaryotic state and selection pressures of cultural conditions would lead to homokaryotic, non-sporulating culture. Heterokaryosis is well established in *Pyricularia* (Suzuki, 1965, 1967) and this would seem to account for the varied sporulation response of *Pyricularia* on detached leaves as well.

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